Novel Endotypes in Heart Failure: Effects on Guideline-Directed Medical Therapy

J. Tromp*, MD (1), W. Ouwerkerk*, MSc (2), B. G. Demissei, MD, PhD (1), S.D. Anker, MD, PhD (3, 4), J.G. Cleland, MD (5), K. Dickstein, MD, PhD (6), G. Filippatos, MD (7), P. van der Harst, MD, PhD (1), H.L. Hillege, MD, PhD (1), C.C. Lang, MD (8), M. Metra, MD (9), L.L. Ng, MD (10), P. Ponikowski, MD (11) N.J Samani, MD (10), D.J. van Veldhuisen, MD, PhD (1), F. Zannad, MD, PhD (12), A.H. Zwinderman, PhD (2), A.A. Voors MD, PhD (1), and P. van der Meer MD, PhD (1).

*These authors contributed equally.

1. Department of Cardiology, University of Groningen, Groningen, the Netherlands

2. Department of Epidemiology, biostatistics & bioinformatics, Academic Medical Center, Amsterdam, The Netherlands

3. Division of Cardiology and Metabolism – Heart Failure, Cachexia & Sarcopenia; Department of Cardiology (CVK); and Berlin-Brandenburg Center for Regenerative Therapies (BCRT), at Charité University Medicine, Berlin, Germany

4. Department of Cardiology and Pneumology, University Medicine Göttingen (UMG), Göttingen, Germany & DZHK (German Center for Cardiovascular Research)

5. National Heart & Lung Institute, Royal Brompton & Harefield Hospitals, Imperial College, London, United Kingdom.

6. University of Bergen, Stavanger University Hospital, Stavanger, Norway

7. National and Kapodistrian University of Athens, School of Medicine, Department of Cardiology, Heart Failure Unit, Athens University Hospital Attikon, Athens, Greece

8. Division of Molecular & Clinical Medicine, University of Dundee, Dundee DD1 9SY, UK

9. Institute of Cardiology, Department of medical and surgical specialties, radiological sciences and public health; University of Brescia, Italy

10. Department of Cardiovascular Sciences, University of Leicester, Glenfield Hospital, Leicester, UK and NIHR Leicester Biomedical Research Centre, Glenfield Hospital, Leicester, LE3 9QP, UK

11. Department of Heart Diseases, Wroclaw Medical University, Poland and Cardiology Department, Military Hospital, Wroclaw, Poland.

12. Inserm CIC 1433, Université de Lorrain, CHU de Nancy, Nancy, France

Word count abstract: 250

Word count manuscript (including references, tables and figure legends): 4986

Abstract

Background: We sought to determine subtypes of patients with heart failure (HF) with a distinct clinical profile and treatment response, using a wide range of biomarkers from various pathophysiological domains.

Method and results: We performed unsupervised cluster analysis using 92 established cardiovascular biomarkers to identify mutually exclusive subgroups (endotypes) of 1802 patients with HF and reduced ejection fraction (HFrEF) from the BIOSTAT-CHF project. We validated our findings in an independent cohort of 813 patients.

Based on their biomarker profile, six endotypes were identified. Patients with endotype 1 were youngest, less symptomatic, had the lowest NT-proBNP levels and lowest risk for all-cause mortality or hospitalization for HF. Patients with endotype 4 had more severe symptoms and signs of HF, higher NT-proBNP levels and were at highest risk for all-cause mortality or hospitalization for HF (HR 1.4; 95%CI 1.1-1.8). Patients with endotypes 2, 3 and 5 were better up-titrated to target doses of beta-blockers ($p<0.02$ for all). In contrast to other endotypes, patients with endotype 5 derived no potential survival benefit from uptitration of ACEi/ARB and beta-blockers ($P_{interaction}$ <0.001). Patients with endotype 2 (HR 1.29; 95%CI 1.10-1.42) experienced possible harm from uptitration of beta-blockers in contrast to patients with endotype 4 and 6 that experienced benefit ($P_{interaction}$ for all <0.001). Results were strikingly similar in the independent validation cohort.

Conclusion: Using unsupervised cluster analysis, solely based on biomarker profiles, six distinct endotypes were identified with remarkable differences in characteristics, clinical outcome, and response to uptitration of guideline directed medical therapy.

Abbreviations.

HF: Heart failure

ACEi: ACE-Inhibitor

ARB: Angiotensin receptor blockers

CKD: Chronic kidney disease

LVEF: Left ventricular ejection fraction

BNP: B-type natriuretic peptide

NT-proBNP: N-terminal pro-B-type natriuretic peptide

Introduction

Heart failure (HF) is associated with considerably high rates of mortality and morbidity[1,2]. The etiology and pathophysiology of HF show substantial interindividual heterogeneity[3–5]. Nevertheless, patients with HF are uniformly treated according to guidelines with ACE-inhibitors (ACEi) and beta-blockers[6,7]. Distinguishing relevant disease subtypes within the spectrum of patients with HF is imperative to create a better understanding of the underlying pathophysiology as well as to identify subgroups of patients not benefiting from available treatment options. Clustering algorithms are frequently used to identify subgroups. Clustering methods try to identify mutually exclusive subgroups based on a set of variables. Recently, Ahmad et al. showed distinct disease phenotypes with differing outcomes by using a cluster-based approach[4]. However, the use of clinical characteristics as the basis for subgroup determination has been criticized, since this will yield naturally occurring clusters of signs and symptoms and not distinct disease subtypes[8]. The advantage of using biomarker profiles over clinical characteristics to determine cluster membership, is that it enables us to possibly identify patients who phenotypically look the same, yet might respond differently to guideline directed medication based on their underlying biomarker profile.

Therefore, we aimed to identify mutually exclusive subtypes of HF patients based on biomarker profiles using a wide range of cardiovascular biomarkers, which can provide new insights into the heterogeneity of HF. These endotypes are then compared with regards to their characteristics, clinical outcome, and their benefit/harm to uptitration of ACEi/angiotensin receptor blockers (ARBs) and/or beta-blockers.

Methods.

Patient population.

This study utilized patients from the BIOSTAT-CHF project, which is described elsewhere[9]. In short, the BIOSTAT-CHF study includes two cohorts of patients with HF. The index cohort consists of 2516 patients with HF from 69 centers in 11 European countries. Inclusion criteria for the index cohort include: patients with >18 years of age, having symptoms of new-onset or worsening HF, confirmed either by a left ventricular ejection fraction (LVEF) of ≤40% or B-type natriuretic peptide (BNP) and/or N-terminal pro-B-type natriuretic peptide (NT-proBNP) plasma levels >400 pg/ml or >2,000 pg/ml, respectively. Patients had not been previously treated with an ACEi/ARBs and/or beta-blocker or they received ≤50% of ACEi/ARB and/or beta-blockers at the time of inclusion and anticipated initiation/up-titration of ACEi/ARBs and beta-blockers.

The validation cohort includes 1738 patients from 6 centers in Scotland, UK. Patients were required to be ≥18 years of age, diagnosed with HF and were previously admitted with HF requiring diuretic treatment. They were sub-optimally treated with ACEi/ARBs and/or beta-blockers, and anticipated initiation or uptitration of ACEi/ARBs and beta-blockers. Patients in both cohorts could be enrolled as in-patients or from out-patient clinics[9].

Of the 2,516 patients included in the index cohort, we excluded 151 patients who died and 23 patients who were censored before 3 months follow-up. Additionally, we excluded 242 patients with LVEF>40%. Of the remaining 2,100 patients, there were 298 patients with missing values on the biomarkers. Subsequent analyses were done with data from the remaining 1,802 patients[10].

Findings were validated in 813 patients with LVEF ≤40% and biomarker measurements available in the validation cohort.

Clinical measurements and definitions.

Medical history, medication use and physical examination were recorded at baseline. Changes in ACEi/ARBs and beta-blockers were recorded. Investigators were expected to optimize treatment within the first 3 months. Patients were considered successfully up-titrated when recommended dose for either ACEi/ARB or beta-blocker was achieved after 3 months of uptitration according to current ESC guidelines[6]. The achieved dose was defined as the highest dose achieved within the uptitration period in percentage of the recommended treatment dose for either ACEi/ARB or beta-blocker.

Outcome analyses

To investigate possible differences between endotypes and outcome, we used a combined the combined outcome of all-cause mortality and HF hospitalizations at 2 years. Hospitalizations due to HF were determined by the investigator. We investigated whether a difference in treatment response could be observed between endotypes. Treatment response is defined as the survival benefit of successful uptitration to guideline directed target dosages for the combined outcome.

Biomarker measurements.

An overview of biomarkers and their pathophysiological function are presented in *supplementary table 1*. Biomarkers were measured using the Olink Proseek® Multiplex CVD III^{96x96} kit. The kit uses a proximity extension assay (PEA) technology, where 92 oligonucleotide-labeled antibody probe pairs bind to their respective targets. When bound, antibodies with DNA reported molecules give rise to new DNA amplicons each ID-barcoding their respective antigens. These amplicons are quantified using a Fluidigm BioMark™ HD real-time PCR platform. The platform provides normalized protein expression (NPX, log2-normalized), but not an absolute quantification. In total, 98.4% of measurements were within range, 1.6% of measurements were below the lower limit of detection (LOD). These were replaced by the LOD, which was found reasonable when having less than 10% of measurements below the LOD[11,12]. Characteristics of the biomarker assay are presented in *supplementary table 2*.

Statistical analysis.

We have provided a comprehensive explanation of the statistical methods used in the *supplementary material*. In brief, the primary analytical goal of this study is to identify mutually exclusive subgroups of patients (clusters) based on their biomarker profile using 92 biomarkers, which we have called endotypes. Biomarker dimensions were reduced by performing principal component analysis (PCA). The optimal number of clusters in our analyses was determined using the package NBclust in R. The package NBclust uses a wide array of different measures to select the optimal number of clusters in a given dataset. Following, the number of cluster most often selected throughout is then selected as the optimal number of clusters for the analyses[13]. We have used k-nearest neighbors to validate our findings[3,14–16]. Cluster membership in the validation

cohort was determined by first projecting the results of the PCA on the biomarker in the validation cohort, followed by the calculation of the nearest cluster, using k-nearest neighbors in the index cohort, for each patient in the validation cohort[14–16].

Differences between clinical characteristics of endotypes were compared using one-way analysis of covariance (ANOVA), the Kruskal-Wallis test or the chi2-test where appropriate. Differences of biomarkers means between endotypes were plotted using a heatmap after z-standardization of biomarker means to make them comparable. The C-index for the 3 biomarkers with the lowest p-value for association with individual clusters were assessed.

The association with the primary outcome was investigated using Kaplan-Meier curves and the log-rank test. For multivariable analyses, Cox regression analysis was performed, correcting for relevant clinical confounders and the BIOSTAT risk model, which was previously published[17]. The BIOSTAT risk model for predicting mortality included, age, blood urea nitrogen (BUN), N-terminal NT-proBNP, hemoglobin and the use of a beta-blocker at time of inclusion. The BIOSTAT risk model for predicting mortality or HF hospitalization included age, NT-proBNP, hemoglobin, the use of a beta-blocker at time of inclusion, a HF-hospitalization in year before inclusion, peripheral edema, systolic blood pressure, high-density lipoprotein cholesterol and sodium.

The association between endotypes and uptitration rates of ACEi/ARBs and beta-blockers to recommended target doses was investigated using logistic regression and corrected for the previously published uptitration models from the BIOSTAT cohort[18]. For ACEi/ARB this model includes sex, BMI, eGFR, alkaline phosphate and country. For beta-blockers, this model included age, country of origin, diastolic blood pressure, heart rate and pulmonary congestion at baseline. Additionally, we have corrected for important clinical confounder including ischemic etiology,

potassium levels and use of MRAs at time of inclusion. To investigate a difference in treatment benefit of being uptitrated to guideline directed medication levels during follow up, we performed interaction analysis between endotype membership and being uptitrated to ≥100% of guideline recommended dosages (yes vs. no) or ACEi/ARB or beta-blockers. To adjust for treatment-indication bias, risk estimates for the primary endpoint for successful uptitration of ACEi/ARB and beta-blockers were adjusted using inverse probability weighting using 55 clinical and laboratory variables (*supplementary table 3*).

Results.

Clustering outcomes.

The optimal number of clusters was 6, ranging from a minimum of 80 to a maximum of 435 patients (*supplemental figure 2*). Heatmaps of biomarkers across endotypes for the index and validation cohort are depicted in *figure 1,* and C-indexes of the top 3 significantly associated biomarkers per endotype presented in *table 1* (validation in *supplementary table 4*). Overall, a limited number of biomarkers identified endotype membership with a relatively high C-index (≥0.78; *table 1*). Patients with endotype 5 had very low levels of chitotriosidase 1 (CHIT1).

Clinical Characteristics.

Baseline characteristics of subgroups are presented in *table 2.* Patients with endotype 1 were youngest, more often in NYHA class I/II (58%) and had relatively mild signs and symptoms

compared to patients with other endotypes. Patients with endotype 1 had the lowest rates of anemia and lowest NT-proBNP levels. Patients with endotype 2 had the higher rates of anemia (45.1%) and high rates of CKD (65.4%) compared to other endotypes (P <0.001). Patients with endotype 3 most often had an ischemic etiology of HF. Patients with endotype 4 had the worst signs and symptoms and highest NT-proBNP levels. Patients with endotype 5 had relatively high rates of anemia (40%). Patients with endotype 6 had the highest rates of hypertension (66%). A summary of clinical characteristics per endotype is provided in *supplementary figure 1*.

Outcome.

After a median follow-up of 21 months, (34%) patients either had a hospitalization for HF or died. Event rate was highest in endotype 4 (48%) and lowest in endotype 1 (24%) (*figure 2*). Compared to the endotype with the best clinical outcome (endotype 1), patients with endotype 4 had the worst outcomes for both the primary combined outcome (HR1.8; 95%CI [1.2-2.7]) and for all-cause mortality alone (HR2.5; 95%CI [1.4-4.5]). After correction for the BIOSTAT-CHF risk models, endotype 4 had worse outcomes compared to endotype 1 for the combined outcome, while endotypes 2 and 4 had higher rates of mortality alone (*table 3; supplementary table 5*). Compared to the BIOSTAT-CHF risk model (C-index 0.71), the classification into endotypes performed worse (C-index 0.61). Interestingly, the BIOSTAT-CHF risk model performed worse in endotypes 2, 3 and 4 (C-index~ 0.64) and better in endotypes 6 (C-index 0.75; *supplementary table 6*).

Uptitration of HF medication to guideline directed dosages and treatment response.

Overall rates of uptitration to recommended target dose of ACEi/ARBs were lowest in endotype 4 and highest in endotypes 3 and 6 (*figure 3A*). Significantly less benefit was observed for uptitration of ACEi/ARB uptitration for endotype 5 (HR 1.29; 95%CI [0.88-1.88]) for the primary combined outcome (*figure 3B, supplementary table 7,* $P_{interaction}$ <0.001).

Beta-blocker uptitration rates was lowest in endotype 6 and highest in endotypes 1 and 5, also after correction for ACEi/ARB uptitration rates (p <0.01 *figure 3C*). Endotype 6 derived more benefit from successful uptitration on beta-blockers for the combined outcome. In contrast, endotype 2 (HR 1.29; 95%CI [1.10-1.52]) had a negative treatment response to beta-blocker uptitration, while endotype 5 did not seem to derive any benefit (*figure 3D, supplementary table 7,* $P_{interaction}$ <0.001).

Validation.

Patients in the validation cohort were older with lower NT-proBNP levels, other characteristics were generally comparable between both cohorts (*supplementary table 8*).

Overall, the results of the cluster analysis were remarkably similar between the index and the validation cohort. Particularly the relative differences between clusters were well validated between cohorts. Figure 1 shows the marked similarity in the biomarker profiles between both cohorts. *Supplementary table 9* shows the great similarity in clinical characteristics of the 6 endotypes between both the index and validation cohorts. Figure 2 shows the remarkable similarity in clinical outcome: endotype 4 had the worst outcomes and patients with endotype 1 had the best outcomes of all endotypes.

Discussion.

Using sophisticated classification techniques based on biomarker profiles, novel mutually exclusive subgroups in HF were identified and validated in an independent cohort. We found striking differences between endotypes in terms of mortality and/or HF hospitalization, uptitration rates of guideline-directed medication, and treatment response. These data show that when classifying patients based on biomarker profiles, specific subgroups with a heterogeneous clinical profile emerge. These specific "endotypes" are not only different in terms of their clinical profile, but also with regards to clinical outcome and their response to uptitration of ACEi/ARB and beta-blockers. This is the first study using a large panel of biomarkers to identify subgroups in HF.

Previous studies in HF identified subgroups via cluster analysis using clinical characteristics, echocardiographic variables and laboratory data[3,4]. A study by Ahmed et al. found novel subgroups in patients with HFrEF using clinical characteristics, however it was suggested that this study potentially identified subgroups based on disease severity and not actual subtypes based on differences in underlying disease mechanisms[4]. Of note, Shah et al. identified phenotypes of patients with HFpEF using clinical characteristics, echocardiographic parameters and laboratory data, which could reflect underlying pathophysiological differences more directly[3]. The present study solely used biomarker profiles for defining subgroups in HF using a comprehensive set of biomarkers reflecting a greater number of disease domains. The dynamic state of biomarkers suggests that not all biomarker levels reflect a consistent biological response, but instead a snapshot of the biological processes at that time point. Here, PCA can reclassify biomarkers into individual biological processes, which reduces the dynamic effect of individual biomarkers[19,20]. Future studies should focus on parameters reflecting a more consistent biological response. A

potential strength of using biomarker profiles to reclassify patients with HF, is that we were able to identify patients with a specific endotype, who might have a non-remarkable phenotype based on clinical variables but respond differently to guideline-directed treatment. An important case-in-point of this, is endotype 2. Patients with this endotype did not show a strong phenotype, yet these patients seemingly did not derive treatment benefit from beta-blockers treatment at guideline directed levels.

The 6 endotypes identified had a distinct biomarker profile and phenotype. A possible important difference was observed for patients with endotype 1 (best outcomes) and patients with endotype 4(worst outcomes). Patients with endotype 1 had very low levels of IGFBP1 and NT-proBNP, while patients with endotype 4 had very high levels of IGFBP1 and NT-proBNP. The very low levels of CHIT1 found in patients with endotype 5 were striking. CHIT1, part of a family of hydrolyzing enzymes, is active in both pathophysiological as well as in physiological circumstances[21]. Increased levels of CHIT1 are associated with arteriosclerosis and Gaucher's disease, furthermore 10-25% of European populations are CHIT1 deficient due to a genetic polymorphism[22]. Interestingly, endotype 5 was deficient for CHIT1 and constituted roughly 4% of the patients in this index cohort. This suggest that CHIT1 might be an interesting novel target, which deserved further study. A limited number of biomarkers could adequately discriminate patient endotype membership with a high C-index. This suggests that in a clinical setting, a patient's endotype membership can be determined by measuring a relatively small number of biomarkers. While promising, more work needs to be done to increase clinical feasibility and cost-effectiveness of this method.

While endotype membership was an independent predictor of outcome, the overall goal of cluster analysis and this study was not to provide a novel prediction model based on endotypes.

There are more advanced techniques to improve risk stratification using both unsupervised as well as supervised techniques, including neural network analysis and support vector machine[23]. Instead, the goal of this study was to provide for a novel classification of HF patients by identifying mutually exclusive subgroups based on biomarker profiles. These subgroups can then potentially be used to optimize risk stratification. Indeed, our results show that there are clear differences in the C-index of the BIOSTAT-CHF risk model between subgroups[17]. Hence, (re-)classification of patients with HF, might improve risk stratification using existing risk prediction models.

There were marked differences in the uptitration rates of ACE/ARB and beta-blockers, particularly patients with endotypes 3 and 6 were more often uptitrated to target dose for ACEi/ARB and patients with endotypes 1and 5 were more often uptitrated to target dose for beta-blockers, independent of confounders. Patients with endotype 2 seemed to derive more benefit of ACEi/ARB uptitration than other endotypes. This is of particular interest given the high rates of CKD in patients with endotype 2. There is a paucity of data on the benefits of ACEi/ARB usage in patients with CKD and HF, due to exclusion of these patients in most randomized controlled trials[24–27]. Patients with endotype 2 derived potential harm from uptitration to guideline directed dosages of beta-blockers. This suggests that beyond clinical characteristics, the endotype of a patient might determine response to guideline-directed medication.

This study has important implications. Firstly, using biomarker profiles to group HF patients leads to potentially clinically meaningful subgroups in HF with differences in uptitration rates as well as treatment benefit of key HF guideline medications independent of confounders. Therefore, patients with similar phenotypes, may respond differently to guideline-directed medication based on their respective endotype, which deserver further study. Furthermore, we observed that subgroup membership could be identified with relatively high C-indexes using single

biomarkers. This suggests that in a clinical setting, a small set of biomarkers can be used to identify a patient's subgroup membership.

Limitations.

First of all, biomarkers used were part of a cardiovascular disease panel, which might not completely reflect the pathophysiological processes within HF. Secondly, we tried to correct for indication bias by performing inverse-probability-weighting, but it cannot be established whether we corrected sufficiently for indication bias. Additionally, the BIOSTAT-CHF is primarily a Caucasian cohort, extrapolation of results to other ethnicities is unclear. Pharmacological therapy at time of study inclusion might have influenced plasma levels of some biomarkers, which could not be accounted for in the analyses. As per design, information on uptitration was not available in the validation cohort. No absolute biomarker levels were available. Despite rigorous statistical techniques to correct for indication bias, results of this study might be further confounded by indication bias and need to be repeated in a more controlled setting. Lastly, echocardiography was not an entry criterion for the BIOSTAT-CHF and echocardiography was performed within 2 years before baseline.

Conclusions.

This is the first study performing a comprehensive cluster analysis in patients with HF based on a large panel of biomarkers Our data suggest that specific pathophysiological profiles, reflected by

circulating biomarkers, have a differential impact on clinical outcome and the response to uptitration of ACEi/ARB and beta-blockers.

Acknowledgements.

BIOSTAT-CHF was funded by the European Commission [FP7-242209-BIOSTAT-CHF; EudraCT 2010-020808-29].

Disclosures.

SDA received grants from Vifor, Abbott Vascular, consultancy or speaking from Vifor, Bayer, Boehringer Ingelheim, Brahms, Janssen, Novartis, Servier, and Stealth Peptides, and ASTRA. MM received consulting or speaker fees from Amgen, Astra-Zeneca, Bayer, Novartis, Relypsa, Servier, Stealth therapeutics, Trevena, Abbott Vascular. GF has received committee fees and/or research grants from Novartis, Bayer, Vifor, Servier. AAV received consultancy fees and/or research grants from Alere, Amgen, Bayer, Boehringer Ingelheim, Cardio3Biosciences, Celladon, GSK, Merck, Novartis, Servier, Stealth Peptides, Singulex, Sphingotec, Trevena, Vifor, and ZS Pharma. CCL received fees and/or research grants from Novartis, Astra Zenenca and MSD All other authors have nothing to disclose.

References.

1. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. *N Engl J Med* Cardiorenal Research Laboratory, Mayo Clinic College of Medicine, Rochester, Minn 55905, USA.: Massachusetts Medical Society; 2006;**355**:251–259.

2. Lam CSP, Donal E, Kraigher-Krainer E, Vasan RS. Epidemiology and clinical course of heart failure with preserved ejection fraction. *Eur J Heart Fail* 2011;**13**:18–28.

3. Shah SJ, Katz DH, Selvaraj S, Burke MA, Yancy CW, Gheorghiade M, Bonow RO, Huang C-C, Deo RC. Phenomapping for novel classification of heart failure with preserved ejection fraction. *Circulation* 2015;**131**:269–279.

4. Ahmad T, Pencina MJ, Schulte PJ, O'Brien E, Whellan DJ, Piña IL, Kitzman DW, Lee KL, O'Connor CM, Felker GM. Clinical implications of chronic heart failure phenotypes defined by cluster analysis. *J Am Coll Cardiol* Journal of the American College of Cardiology; 2014;**64**:1765–1774.

5. Shah AM, Solomon SD. Phenotypic and pathophysiological heterogeneity in heart failure with preserved ejection fraction. *Eur Heart J* Oxford University Press; 2012;**33**:1716–1717.

6. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, González-Juanatey JR, Harjola V-P, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GMC, Ruilope LM, Ruschitzka F, Rutten FH, Meer P van der. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur J Heart Fail* 2016;**18**:891–975.

7. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Drazner MH, Fonarow GC, Geraci SA, Horwich T, Januzzi JL, Johnson MR, Kasper EK, Levy WC, Masoudi FA, McBride PE,

McMurray JJV, Mitchell JE, Peterson PN, Riegel B, Sam F, Stevenson LW, Tang WHW, Tsai EJ, Wilkoff BL. 2013 ACCF/AHA Guideline for the Management of Heart Failure. *Circulation* 2013;**128**.

8. Francis GS, Cogswell R, Thenappan T. The Heterogeneity of Heart Failure. *J Am Coll Cardiol* 2014;**64**.

9. Voors AA, Anker SD, Cleland JG, Dickstein K, Filippatos G, Harst P van der, Hillege HL, Lang CC, Maaten JM Ter, Ng L, Ponikowski P, Samani NJ, Veldhuisen DJ van, Zannad F, Zwinderman AH, Metra M. A systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure: rationale, design, and baseline characteristics of BIOSTAT-CHF. *Eur J Heart Fail* 2016;**18**:716–726.

10. Ouwerkerk W, Zwinderman AH, Ng LL, Demissei B, Hillege HL, Zannad F, Veldhuisen DJ van, Samani NJ, Ponikowski P, Metra M, Maaten JM ter, Lang CC, Harst P van der, Filippatos G, Dickstein K, Cleland JG, Anker SD, Voors AA. Biomarker-Guided Versus Guideline-Based Treatment of Patients With Heart Failure. *J Am Coll Cardiol* 2018;**71**:386–398.

11. Croghan CW. Methods of Dealing with Values Below the Limit of Detection using SAS.

12. Verbovšek T. A comparison of parameters below the limit of detection in geochemical analyses by substitution methods Primerjava ocenitev parametrov pod mejo določljivosti pri geokemičnih analizah z metodo nadomeščanja. *RMZ – Mater Geoenvironment* 2011;**58**:393–404.

13. Charrad M, Ghazzali N, Boiteau V, Niknafs A. NbClust : An R Package for Determining the Relevant Number of Clusters in a Data Set. *J Stat Softw* 2014;**61**:1–36.

14. Leisch F. A toolbox for K-centroids cluster analysis. *Comput Stat Data Anal* 2006;**51**:526–544.

15. Leisch F, Grün B. Extending Standard Cluster Algorithms to Allow for Group Constraints *.

16. Hastie T, Tibshirani R, Friedman J. The Elements of Statistical Learning. New York, NY:

Springer New York; 2009.

17. Voors AA, Ouwerkerk W, Zannad F, Veldhuisen DJ van, Samani NJ, Ponikowski P, Ng LL, Metra M, Maaten JM ter, Lang CC, Hillege HL, Harst P van der, Filippatos G, Dickstein K, Cleland JG, Anker SD, Zwinderman AH. Development and validation of multivariable models to predict mortality and hospitalization in patients with heart failure. *Eur J Heart Fail* 2017;

18. Ouwerkerk W, Voors AA, Anker SD, Cleland JG, Dickstein K, Filippatos G, Harst P Van Der, Hillege HL, Lang CC, Maaten JM Ter, Ng LL, Ponikowski P, Samani NJ, Veldhuisen DJ Van, Zannad F, Metra M, Zwinderman AH. Determinants and clinical outcome of uptitration of ACE-inhibitors and beta-blockers in patients with heart failure: A prospective European study. *Eur Heart J* 2017;**38**:1883–1890.

19. Yao F, Coquery J, Lê Cao K-A. Independent Principal Component Analysis for biologically meaningful dimension reduction of large biological data sets. *BMC Bioinformatics* BioMed Central; 2012;**13**:24.

20. Jolliffe I, Jolliffe, Ian. Principal Component Analysis. *Encyclopedia of Statistics in Behavioral Science* Chichester, UK: John Wiley & Sons, Ltd; 2005.

21. Kanneganti M, Kamba A, Mizoguchi E. Role of chitotriosidase (chitinase 1) under normal and disease conditions. *J Epithel Biol Pharmacol* NIH Public Access; 2012;**5**:1–9.

22. Ober C, Chupp GL. The chitinase and chitinase-like proteins: a review of genetic and functional studies in asthma and immune-mediated diseases. *Curr Opin Allergy Clin Immunol* NIH Public Access; 2009;**9**:401–408.

23. Chi C-L, Street WN, Wolberg WH. Application of artificial neural network-based survival analysis on two breast cancer datasets. *AMIA . Annu Symp proceedings AMIA Symp* American Medical Informatics Association; 2007;**2007**:130–134.

24. Effects of Enalapril on Mortality in Severe Congestive Heart Failure. *N Engl J Med* 1987;**316**:1429–1435.

25. Effect of Enalapril on Survival in Patients with Reduced Left Ventricular Ejection Fractions and Congestive Heart Failure. *N Engl J Med* 1991;**325**:293–302.

26. The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II): a randomised trial. *Lancet (London, England)* 1999;**353**:9–13.

27. Packer M, Coats AJS, Fowler MB, Katus HA, Krum H, Mohacsi P, Rouleau JL, Tendera M, Castaigne A, Roecker EB, Schultz MK, Staiger C, Curtin EL, DeMets DL. Effect of Carvedilol on Survival in Severe Chronic Heart Failure. *N Engl J Med* 2001;**344**:1651–1658.

Figure legends.

Figure 1: Heatmap displaying biomarker across endotypes for the index (A) and validation (B) cohort.

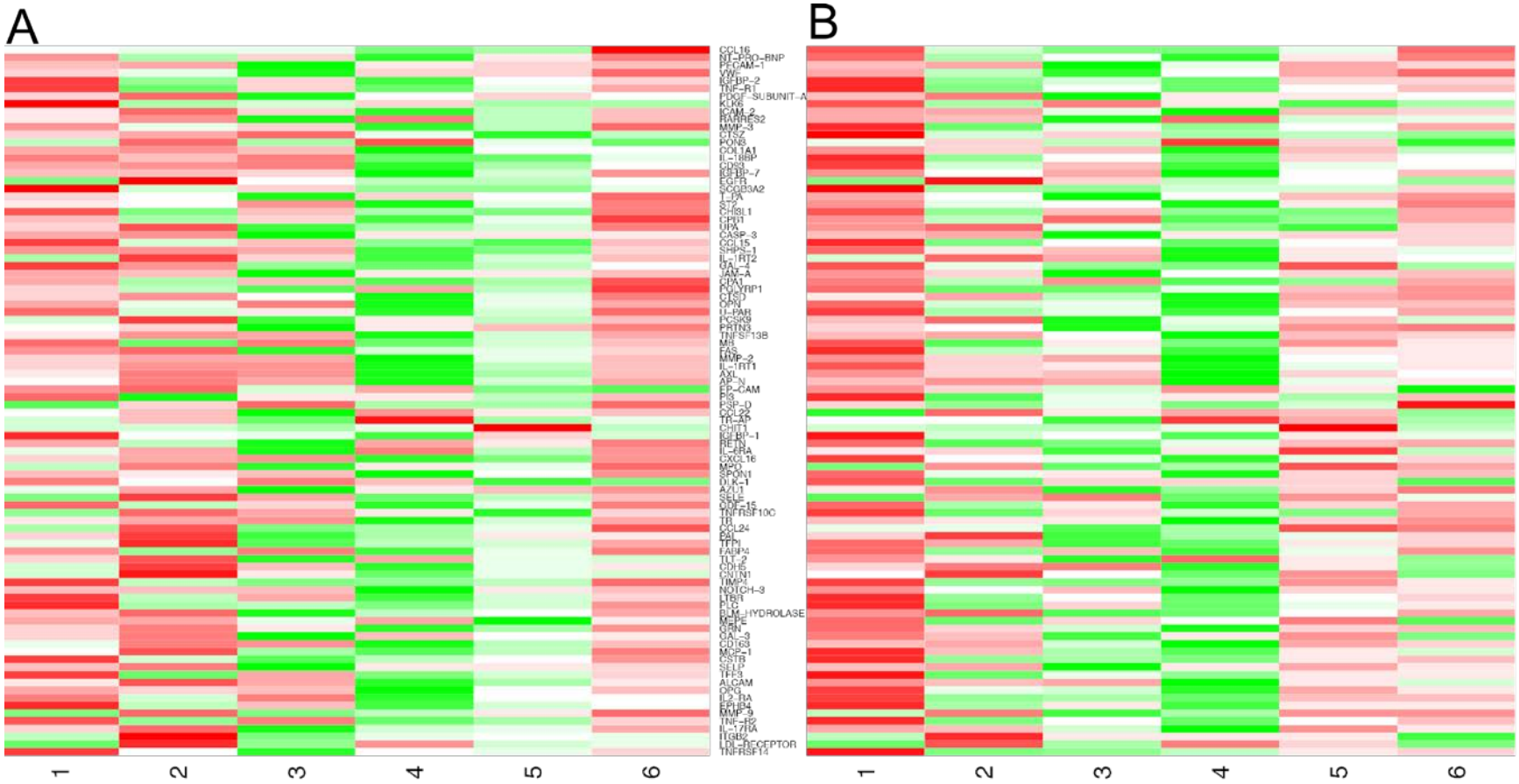

Figure 2: Kaplan-Meier curves for the primary combined outcome of all-cause mortality and/or HF hospitalization at 2 years for the index (A) and validation (B) cohort stratified according to endotypes. The log-rank p-value is <0.001 for both the index (A) and validation (B) cohort.

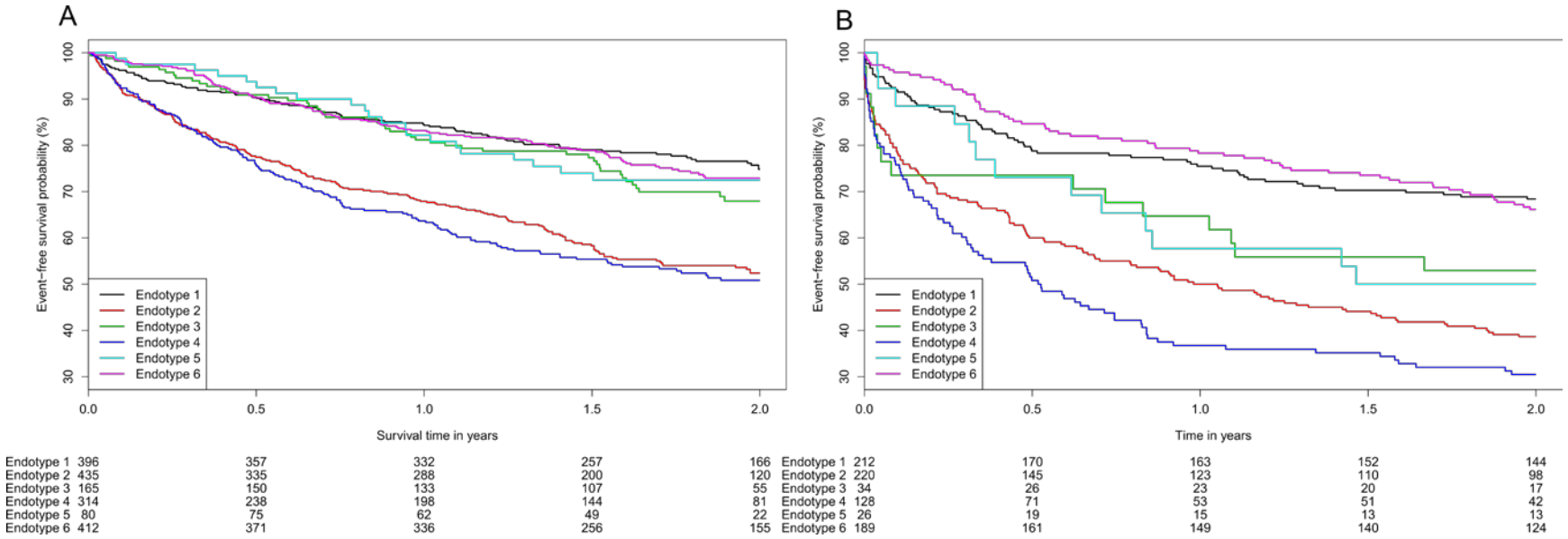

Figure 3: Uptitration rates corrected for the biomarker uptitration model for ACE-inhibitors/ARB (A), beta-blockers (C) and association with outcome of successful uptitration of ACEi/ARB (B) and beta-blockers (D) across endotypes in patients with left ventricular ejection fraction ≤40%.

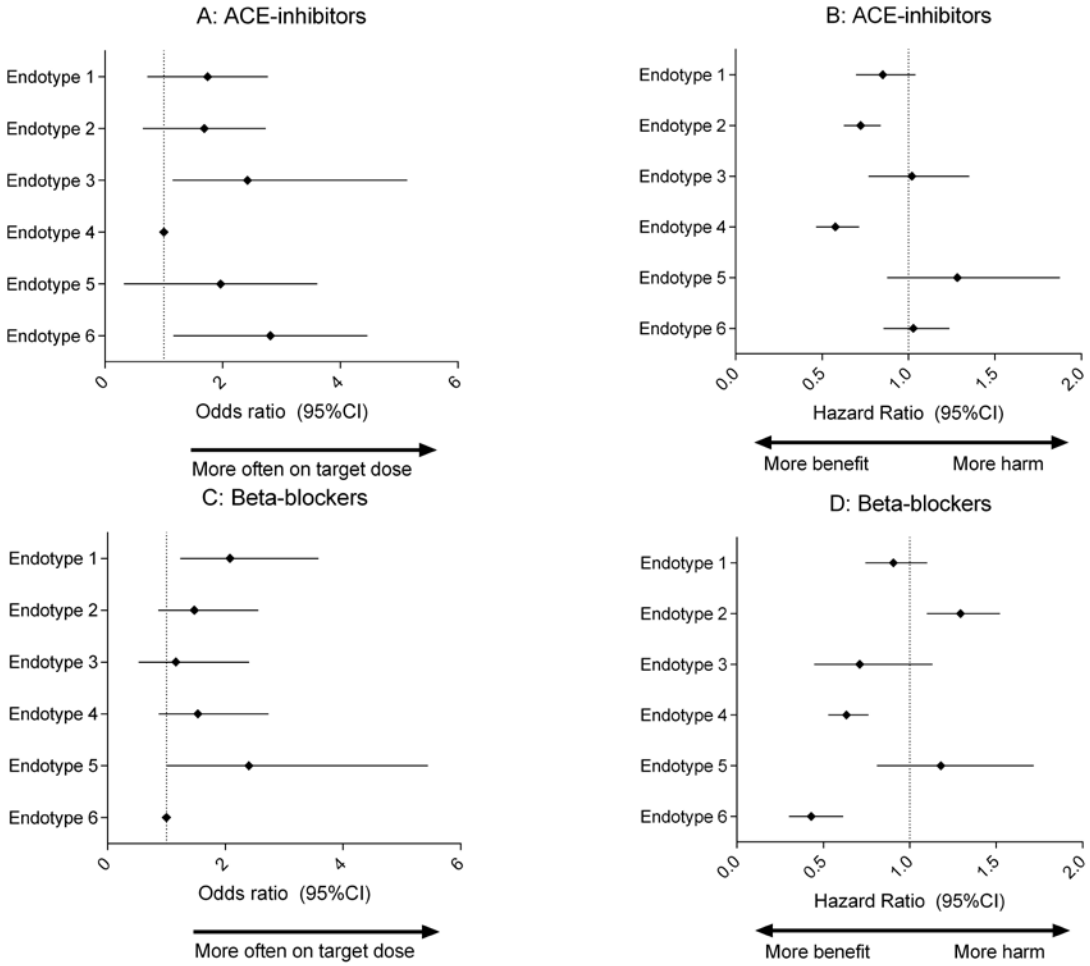

Table 1: Biomarkers subgroup identification.

Endotype 1		**Endotype 2**		**Endotype 3**	
Marker	*C-index*	*Marker*	*C-index*	*Marker*	*C-index*
IGFBP1	0.83	PAI	0.77	SELP	0.93
IGFBP2	0.70	PDGFsA	0.74	PECAM1	0.93
NT-proBNP	0.65	SELP	0.7	JAMA	0.97
Combined	0.83	*Combined*	0.78	*Combined*	0.97

Endotype 4		**Endotype 5**		**Endotype 6**	
Marker	*C-index*	*Marker*	*C-index*	*Marker*	*C-index*
ST2	0.81	CHIT1	0.99	TPA	0.70
NT-proBNP	0.80			NT-proBNP	0.70
IGFBP1	0.80			VWF	0.70
Combined	0.86	*Combined*	NA	*Combined*	0.78

Abbreviations: CHIT1, chitoriosidase-1;IGFBP, insuling-like growth binding factor-binding protein; JAMA, junctional adhesion molecule A; NT-proBNP, N-type pro B-type natriuretic peptide; PAI, Plasminogen activator inhibitor-1; PDFGsA, Platelet-derived growth factor subunit alpha; PECAM1, platelet endothelial cell adhesion molecule; SELP, selectin P ; TPA, tissue-type plasminogen activator; VWF, Von-Willebrand-factor

Table 2: Baseline characteristics.

	Endotype 1	**Endotype 2**	**Endotype 3**	**Endotype 4**	**Endotype 5**	**Endotype 6**	***p-value***
N	**396**	**435**	**165**	**314**	**80**	**412**	
Demographics							
Age(years)	63(12)	73(11)	66(11)	66(13)	66(12)	69(11)	<0.001
Female(%)	82(21%)	104(24%)	35(21%)	73(23%)	13(16%)	133(32%)	<0.001
BMI(kg/m2)	30(6)	27(5)	28(6)	27(5)	28(5)	28(5)	<0.001
Ischemic etiology(%)	165(43%)	207(48%)	82(51%)	119(38%)	37(47%)	203(50%)	0.013
NYHA n(%)							

I	39(10%)	30(7%)	6(4%)	28(9%)	8(10%)	40(10%)	<0.001
II	190(48%)	190 (44%)	88(53%)	120(38%)	40(50%)	221(54%)	
III	102(26%)	129(30%)	48(29%)	117(37%)	21(26%)	109(27%)	
IV	15(4%)	18(4%)	2(1%)	12(4%)	2(3%)	6(2%)	
NA	50(13%)	68(16%)	21(13%)	37(12%)	9(11%)	36(9%)	
Systolic BP(mmHg)	126(22)	122(23)	127(19)	119(21)	125(23)	127(19)	<0.001
Diastolic BP(mmHg)	77(13)	73(14)	77(12)	74(13)	76(16)	76(12)	<0.001
LVEF (%)	29(7)	28(8)	29(8)	26(8)	28(8)	30(7)	<0.001
Heart rate(bpm)	83(22)	80(20)	77(16)	84(21)	81(17)	75(17)	<0.001
Signs and symptoms(%)							
Peripheral edema							
Not Present	159(49%)	126(35%)	79(58%)	55(20%)	34(51%)	192(59%)	<0.001
Ankle	96(30%)	119(33%)	37(27%)	77(29%)	24(36%)	83(26%)	
Below Knee	55(17%)	86(24%)	19(14%)	100(37%)	6(9%)	43(13%)	
Above Knee	14(4%)	25(7%)	1(1%)	38(14%)	3(5%)	5(2%)	
JVP	60(22%)	124(38%)	15(12%)	115(52%)	18(31%)	60(20%)	**<0.001**
Orthopnea	133(34%)	159(37%)	32(19%)	144(46%)	28(35%)	85(21%)	<0.001
Medical history(%)							
Anemia	81(21.8%)	188(45.1%)	36(22.8%)	111(36.5%)	31(40.3%)	116(29.2%)	<0.001
Atrial fibrillation	161(40.7%)	210(48.3%)	64(38.8%)	156(49.7%)	35(43.8%)	147(35.7%)	<0.001
Diabetes	128(32.3%)	132(30.3%)	49(29.7%)	104(33.1%)	24(30.0%)	134(32.5%)	0.94
COPD	55(13.9%)	84(19.3%)	32(19.4%)	56(17.8%)	13(16.3%)	50(12.1%)	0.041
CKD	93(23.5%)	284(65.4%)	61(37.0%)	137(43.6%)	38(47.5%)	179(43.6%)	<0.001
Hypertension	239(60.4%)	256(58.9%)	94(57.0%)	175(55.7%)	43(53.8%)	273(66.3%)	0.046
Medication(%)							
Loop diuretics	394(100%)	433(100%)	165(100%)	313(100%)	80(100%)	409(99%)	0.85
ACEi/ARB	296(75%)	302(69%)	138(84%)	219(70%)	57(71%)	321(78%)	0.002
Betablocker	332(84%)	367(84%)	143(87%)	259(83%)	66(83%)	363(88%)	0.31
MRA	225(57%)	224(52%)	85(52%)	187(60%)	46(58%)	219(53%)	0.23

Laboratory							
Hemoglobin	14(2)	13(2)	14(2)	13(2)	13(2)	13(2)	<0.001
Sodium	140(138, 141)	139(137, 142)	139(137, 141)	139(136, 141)	139(137, 142)	141(138, 142)	<0.001
Potassium	4(4, 5)	4(4, 5)	4(4, 5)	4(4, 5)	4(4, 5)	4(4, 5)	<0.001
NT-proBNP	2570(1315, 3984)	6326(3490, 11809)	3624(1910, 6228)	6181(3360, 10300)	3308(1709, 8797)	2660(1207, 4198)	<0.001

Abbreviations: ACEi, ACE-inhibitor; ARB, angiotensin-II receptor blocker ; BMI, body mass index; BP, blood pressure; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; HF, heart failure; JVP, jugular venous pressure; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NYHA, New York heart association; SBP, systolic blood pressure;

Table 3: Survival analyses.

	Endotype 1	**Endotype 2**	**Endotype 3**	**Endotype 4**	**Endotype 5**	**Endotype 6**
	All-cause mortality and/or Heart failure hospitalizations at 2 years					
	HR (95%CI) p-value	*HR (95%CI) p-value*	*HR (95%CI) p-value*	*HR (95%CI) p-value*	*HR (95%CI) p-value*	*HR (95%CI) p-value*
Univariable	ref	2.3(1.7-2.9) <0.001	1.3(0.9-1.8) 0.171	2.5(1.9-3.2) <0.001	1.1(0.7-1.8) 0.575	1.1(0.8-1.4) 0.563
Model 1	ref	1.9(1.5-2.5) <0.001	1.2(0.9-1.7) 0.266	2.4(1.8-3.1) <0.001	1.1(0.7-1.7) 0.724	1.0(0.8-1.3) 0.939
Model 2	ref	1.5(1.0-2.2) 0.029	1.1(0.6-2.0) 0.760)	1.9(1.3-2.7) 0.002	1.2(0.6-2.5) 0.558	1.3(0.8-1.9) 0.296
Model 3	ref	1.5(1.0-2.2) 0.033	1.1(0.6-2.0) 0.747	1.8(1.2-2.7) 0.003	1.2(0.6-2.4) 0.577	1.3(0.8-1.9) 0.307
BIOSTAT risk model	ref	1.3(1.0-1.7) 0.064	1.2(0.8-1.7) 0.312	1.4(1.1-1.8) 0.019	0.8(0.5-1.3) 0.345	1.0(0.8-1.3) 0.895

Model 1: age & sex; Model 2: model 1 + eGFR, systolic blood pressure, presence of anemia, history of atrial fibrillation and NT-proBNP levels; Model 3: model 2 + fraction target dosages of ACEi/ARB and beta-blockers at 3 months. The BIOSTAT risk model includes: age, blood urea nitrogen, NT-proBNP, hemoglobin levels, usage of beta-blockers at time of inclusion, previous HF hospitalization, presence of peripheral edema, systolic blood pressure, high-density lipoprotein, cholesterol and sodium levels.